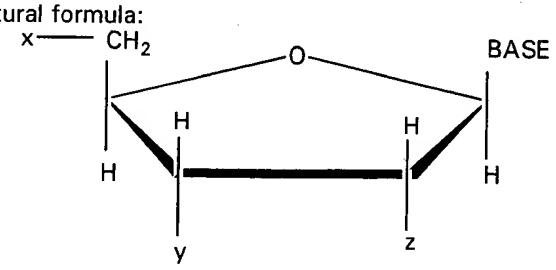
REMARKS

Claims 204-224 and 227-277 were previously pending in this application, and have been canceled hereinabove. Claims 278-453 have been added. Accordingly, 278-453 are being presented for further examination on the merits.

Acknowledgement is made of the fact that the Art Unit location of this application has been changed. All future correspondence will be directed to Examiner Scott Houtteman in Group Art Unit 1809.

By adding a new set of claims in place of the formerly pending claims, Applicants have attempted in a sincere effort to place the claimed subject matter in this application in a significantly better condition for allowance. As indicated above, claims 204-224 and 227-277 have been canceled for new claims 278-453. The new claims are directed in part to a phosphate-modified nucleotide having the formula Sig – PM – SM - BASE (claims 278-301 and 308-309); other compositions comprising at least one such phosphate-modified nucleotide (claims 302-307); an oligo- or polynucleotide comprising at least one nucleotide having the formula Sig – PM – SM - BASE (claims 310-337); and a composition comprising a polymeric compound havng attached directly or indirectly thereto at least one nucleotide having the formula Sig – PM – SM - BASE (claims 338-372). The Examiner will no doubt appreciate that claims 278-372 follow the format of the former and now canceled claims (204-224 and 227-277) in that the Sig – PM – SM - BASE formula are recited in each independent claim (claims 278, 310 and 338).

In the other newly added claims, 373-453, Applicants have elected to describe their claimed invention in more conventional and precise chemical terms, relying on the pentose ring of the sugar moiety with attachments of the other moieties (base moiety, phosphate moiety and Sig moiety) indicated or otherwise recited in the claim language. Thus, claims 373-404 are directed to a nucleotide having the structural formula:



In that structural formula above and in the claim language of claim 373, BASE represents a moiety selected from the group consisting of a pyrimidine, a purine, and a deazapurine, or analog thereof. BASE is attached to the 1' position of the pentose ring from the N1 position when BASE is a pyrimidine or from the N9 position when BASE is a purine or a deazapurine. The x and y groups are selected from H– , HO– , a di-phosphate and a tri-phosphate; whereas z is selected from the group consisting of H– and HO–. Sig is covalently attached to x, y or z directly or through a chemical linkage, and it represents a moiety capable of non-radioactive detection when so attached to x, y or z. Finally, the nucleotide is capable of being incorporated into an oligo- or polynucleotide.

Similarly, claim 405-432 are directed to an oligo- or polynucleotide comprising at least one nucleotide having the structural formula of the pentose ring sugar moiety described above. In the case of those claims, BASE is a moiety selected from the group consisting of a pyrimidine, a purine and a deazapurine, or analog thereof, and it is attached to the 1' position of the pentose ring from the N1 position when a pyrimidine, or from the N9 position when a purine or a deazapurine. Again, x and y are selected from H– , HO– , a mono-phosphate, a di-phosphate and a tri-phosphate.; whereas z is selected from H– and HO–. Sig is covalently attached to x, y or z directly or through a chemical linkage, and it represents a moiety capable of non-radioactive detection when so attached to x, y or z.

In new claims 433-453 is recited a composition comprising a polymeric compound attached directly or indirectly to at least one nucleotide having the structural formula of the pentose ring sugar moiety described above. In the case of this composition, BASE, x, y, z and Sig are as described earlier in the oligo- or polynucleotide claims of 405-432.

With some exceptions, most of the dependent claims being presented now were in the former claims that have been canceled. One exception is the embodiment found in dependent claims 279, 311, 339, 374, 406 and 434, all of which recite "wherein Sig is or renders the nucleotide [or the oligo- or polynucleotide or composition] self-signaling or self-indicating or self-detecting." Support for the "self-signaling or self-indicating or self-detecting" nature of Sig is found in the specification, page 82, first paragraph; in particular, page 95, first

paragraph; page 96, first paragraph; and originally filed claims 141 and 143. Another exeception is claim 332 which depicts the structural formula of the oligo- or polynucleotide comprising at least one phosphate-modified nucleotide. The structural formula in claim 332 is taken from pages 5 and 23. Other execeptions are claims 335-337 that depend (at least ultimately) from the oligo- or polynucleotide of claim 310. Claim 335 recites that the "Sig moiety is attached to a terminal nucleotide in said oligo- or polynucleotide." Support for the quoted language is found in the specification, page 25, second paragraph ("Also, the compounds can be prepared by terminal addition to oligo- or polynucleotides to produce compounds in which m or n is 0 depending upon whether the addition is at the 5' or 3' position"); and page 99, last paragraph, through page 100. Claim 336 depends from claim 335 and recites "wherein the sugar moiety of said terrninal nucleotide has a hydrogen at the 2' position thereof." Support for the foregoing language is also found in the originally filed specification, page 12, third paragraph (particularly lines 10-13 from the bottom of the page: "More likely at least one of x, y, and z will be . . . and at least one will be HO- or H-. As will be readily appreciated, the most likely identity of z will be HO- or H- . . ."); page 15, lines 1-5; and page 24, line 3. Claim 337 also depends from claim 335 and recites "wherein the sugar moiety of said terminal nucleotide has a hydrogen at each of the 2' and 3' positions thereof." Support for the subject matter of claim 337 is likewise taken from the specification, page 12, third paragraph (lines 10-13 from the bottom of the page); and page 15, lines 1-5.

It is believed that none of the new claims, 278-453, constitutes the insertion of new matter into the disclosure. Entry of the above amendments to the claims is respectfully urged.

Before addressing the substantive issues in the June 25, 1997 Office Action, Applicants and their undersigned attorney appreciate the courtesy extended by the Examiner to John J. Santalone, Esq., another attorney of record, in a telephone conversation during the week of September 22nd - September 26th. Examiner Houtteman indicated that he would grant extensions of time for responding to the Office Action, notwithstanding the notice given in the cover sheet dated June 25, 1997.

Request Under 37 C.F.R. §1.129(a) for Withdrawal of the Finality of the June 25, 1997 Office Action

Applicants wish to avail themselves of the provision of 37 C.F.R. §1.129(a) by requesting the withdrawal of the finality of the June 25, 1997 Office Action. Accordingly, Applicants are filing concurrently with this Amendment a Request Under 37 C.F.R. §1.129(a) for Withdrawal of the Finality of the June 25, 1997 Office Action. As suggested by the Examiner in that Office Action, the finality of the Office Action will be withdrawn upon timely filing of a first submission and the appropriate fee, that is, $770 for a large entity under 37 C.F.R. §1.17(r).

Before turning to the other issues in the June 25, 1997 Office Action, Applicants appreciate the indication from the Examiner that the objection and rejections of paragraphs 16A and 16B in the June 21, 1996 Office Action have been withdrawn in view of the previous amendments to the claims.

The Objection and Rejection Under 35 U.S.C. §112, First Paragraph

The specification stands objected to under 35 U.S.C. §112, first paragraph, because allegedly the specification, as originally filed, does not provide support for the invention as is now claimed. In the Office Action (page 2), the Examiner stated "Since support for these claims was not found where pointed out nor elsewhere in the specification, these claims are considered "new matter" for reasons of record. Claims 207-224 and 227-262, 265 and 267 were also rejected under 35 U.S.C. §112, first paragraph, for the reasons set forth in the above objection to the specification.

In the prior June 20, 1996 Office Action, the Examiner had stated:

> Claims 207-224 and 227-262 are drawn to nucleotides having the "Sig" moiety attached to the phosphate moiety wherein the Sig moiety is limited to one of several molecular classes such as "at least three carbon atoms, a glycosidic linkage moiety, biotin, iminobiotin, ferritin, an antigen, a hapten, an antibody, etc.
>
> Support for these claims was pointed out in original claims 125, 41, 84, 126, 129, 127 and 128. However, these claims are drawn to nucleotides in which the "Sig" moiety is attached to the base.

> The only support that was found in the original disclosure was in a passage on pages 96-97 which begins "By way of summary." This passage defines "Sig" as binding to either base, sugar or phosphate and then defines "Sig" to include the particular products in the newly presented claims. However, there is no explicit description of the various claimed products bound to the phosphate anywhere in the specification. In contrast, the base-linked "Sig" moieties have numerous complex chemical reactions which are necessary to synthesize the various products. These reactions include various solvents, reactants and protecting groups which are necessary so that only the base was modified and not the reactive groups on the sugar or phosphates. Thus, an explicit description of the "phosphate-Sig" reactions would have been expected in order for the skilled artisan to have reasonably concluded that the original disclosure evidenced "possession" of the currently claimed invention.
>
> The objection and rejection are respectfully traversed.

In response, Applicants are submitting herewith attached hereto as Exhibit A the Declaration of Dr. Dean L. Engelhardt in Support of Adequate Description and Enablement.[1] It is submitted that Dr. Engelhardt's knowledge and experience qualify him to make his statement as a person of ordinary skill in the art. As noted in his Declaration (Exhibit A), Dr. Engelhardt is one of the named coinventors and a senior vice-president of the instant assignee. After going over the claimed subject matter in this application and the nature of the two rejections under 35 U.S.C. §112, first paragraph (Paragraphs 4, 5A-5D and 6A-6C in his Declaration), Dr. Engelhardt opines in Paragraph 7 that the originally filed specification supports the instantly claimed subject matter such that a skilled artisan would have reasonably concluded that the original disclosure evidenced possession of the invention currently being claimed. Dr. Engelhardt's Declaration is made by him to substantiate both the support and adequate description in the specification for the claims.

As noted in Dr. Engelhardt's Declaration (Paragraphs 8A and 8B), the discovery of Dr. David C. Ward and his group at Yale University, premised upon "several essential criteria," served as a watershed in nucleic acid labeling and detection because it provided for the first time a reliable means for labeling and

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[1] Although Applicants and their attorney do not acknowledge that a *prima facie* case of inadequate description and lack of enablement has been made, they are nevertheless turning to a declaration from a person skilled in the art in order to establish that this application meets the standards of §112, first paragraph, with respect to both adequate description and enablement. By so doing, Applicants are hopeful that prosecution will be expedited.

detecting nucleic acids non-radioactively. But that discovery was only directed to non-disruptive labeling in the base positions - that is, the positions prescribed by the Ward "essential criteria." In Paragraph 9A of his Declaration, Dr. Engelhardt explains that it was a short time after Ward's discovery that the coinventors unexpectedly discovered that nucleic acid labeling and detection could be extended far beyond and in total contradiction to Ward's discovery and criteria. Their subsequent and unexpected discovery culminated in the filing of the first application in 1982 and flew headlong against Ward because the positions for labeling the nucleic acid now involved the so-called "disruptive" and "semi-disruptive" positions in the base. Even more significantly, the novel labeling and labeled compositions involved not only such positions in the base moiety, but the sugar and phosphate moieties as well. As noted by Dr. Engelhardt in his Declaration, this unexpected discovery with respect to the phosphate moiety is set forth in several portions in the instant specification, including the following:

page 94, last paragraph, through page 95, first paragraph;

page 96, through page 98, first paragraph;

page 103, first full paragraph; and

page 103, last paragraph, continuing through page 106, first paragraph.

Later in Paragraph 9B of his Declaration, Dr. Engelhardt points out that there are no fewer than nine (9) instances where the Sig moiety component is described in the specification as being attached to the phosphate moiety P, the sugar moiety S and/or the base moiety B. Dr. Engelhardt lists the following as those instances:

page 90, last paragraph	. . . and a signalling chemical moiety Sig covalently attached thereto, either to the P, S or B moiety.
page 93, first paragraph	. . . include a chemical moiety Sig covalently attached to the P, S and/or B moieties.
page 96, first paragraph	. . . by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig.
page 98, first paragraph	. . . the Sig component or chemical moiety of nucleotides of this invention can be directly covalently attached to the P, S or B moieties or attached thereto via a chemical linkage or linkage arm . . .
page 103, first full paragraph	. . . and the signalling or self-detecting moiety, Sig, covalently attached to either the P, S or B moieties, as indicated hereinabove, . . .
page 104, first paragraph	. . . nucleotides in accordance with this invention containing the above-described components P, S, B and Sig, . . .
page 105, first paragraph	. . . the nucleotides of this invention include the P, S, B and

	Sig components wherein the Sig is covalently attached to either the P, S or B moieties
page 105, second paragraph	The moiety Sig attached to the special nucleotides of this invention containing the other moieties or components P, S, B provides a site per se for the attachment thereto, the Sig component, . . .
page 106, first paragraph	. . . the special P, S, B and Sig-containing nucleotides of this invention, . . .

In Paragraph 9C of his Declaration, Dr. Engelhardt refers to Example V as additional support for the adequate description in the specification for the claimed subject matter. Dr. Engelhardt notes that this example describes a method for attaching biotin, one of the embodiments for Sig, to the phosphate moiety of a mononucleotide and an oligonucleotide that are coupled to a protein, poly-L-lysine. According to Dr. Engelhardt, using the procedure in Example V in the specification (page 57), the biotinylated poly-L-lysine is coupled to a terminal oxygen of the phosphate moiety or to a terminal phosphorus. Dr. Engelhardt notes that these reaction schemes are set forth in Figure 1 on page 374 in Halloran and Parker, J. Immunol., 96:373 (1966) and he has attached a copy of that publication as Exhibit 1 to his Declaration.

In Dr. Engelhardt's opinion, original claim 143 is telling on the issue of using any of the embodiments of Sig for either the base, sugar or phosphate moieties. That claim, recited in its entirety in Paragraph 9D of Dr. Engelhardt's Declaration, specifically recites that the "nucleotide having covalently attached to the P or S or B moiety a chemical moiety Sig . . ."

In Paragraph 9E, Dr. Engelhardt states that the chemical reactions by which substituents are attached to the oxygen or the phosporus atoms of a phosphate or phosphoric acid moiety in a nucleotide (or an oligo- or polynucleotide or other polymer such as a protein) were known in the art prior to the first filing of this application in 1982. As illustrations, Dr. Engelhardt lists the following sixteen (16) publications:

Reactions involving the oxygen

Goody et al., JACS 93:6252-6257 (1971) [Exhibit 2] disclose a reaction for adding a diphenyl to the oxygen atoms of nucleoside di- and triphosphates. See, for example, Scheme I on page 6253.

Eckstein et al., Biochemistry 14:5225-5232 (1975) [Exhibit 3] disclose guanosine 5'-di- and triphosphate derivatives with modified terminal phosphates in which the following substituents are added to the terminal oxygen: methyl, aminoethyl, acetylaminoethyl and phenyl. See, for example,

the discussion under "*Synthesis of Analogues*" beginning on page 5226, left column, and continuing through page 5228, right column. See also Figure 1 on page 5226.

Armstrong et al., European Journal of Biochemistry 70:33-38 (1976) [Exhibit 4] disclose ATP and UTP analogues modified in the phosphate moieties in which a methyl or a phenyl group is attached to a terminal oxygen. See structures I b) and
I c) on page 33, right column.

Reactions involving the phosphorus

Miller et al., Biochemistry 18:5134-5143 (1979) [Exhibit 5] disclose a series of dideoxyribonucleoside methylphosphonate analogues in which a methylene group is contained in the internucleoside linkage. See, for example, the discussion under "*Preparation of Dinucleoside Methylphosphonates*" beginning on page 5136, right column, and continuing through page 5137.

Miller et al., Biochemistry 20:1874-1880 (1981) [Exhibit 6] disclose the preparation of oligodeoxyribonucleoside methylphosphonates. See, for example, the discussion under "*Preparation of Oligonucleoside Methylphosphonates*" beginning on page 1875, left column, and continuing through the first four lines of the right column. See also Table I at the top of page 1876.

Beaucage et al., Tetrahedron Letters 22:1859-1862 (1981) [Exhibit 7] disclose deoxynucleoside phosphoramidites depicted in structures Ia-d and IIIa-d on page 1859 and structures Ia, II and IIIa on page 1861.

Miyoshi et al., Nucleic Acids Research 8:5491-5505 (1980) [Exhibit 8] disclose the preparation of three oligonucleotides, i.e., hexadecanucleotides in which di- and trinucleotides are used as incoming 3'-phosphodiester units. See, for example, Figure 1 on page 5493.

Gait et al., Nucleic Acids Research 8:1081-1096 (1980) [Exhibit 9] disclose the preparation of oligodeoxyribonucleotides up to 12 units long using phosphotriesters.

Duckworth et al., Nucleic Acids Research 9:1691-1706 (1981) [Exhibit 10] disclose the preparation of heptadecadeoxyribonucleotides using phosphotriesters.

Ohtsuka et al., Tetrahedron Letters 23:3081-3084 (1982) [Exhibit 11] disclose the synthesis of dodecadeoxynucleotides using phosphotriesters.

Gough et al., Tetrahedron Letters 22:4177-4180 (1981) [Exhibit 12] disclose the construction of oligodeoxyribonucleotides using phosphotriesters.

Reactions for coupling nucleic acids to other polymers (e.g., proteins, polysaccharides)

Brutlag et al., Biochemistry 8:3214-3218 (1969) [Exhibit 13] disclose cross-linking deoxyribonucleic acid to histone in nucleohistone using formaldehyde.

Manning et al., Chromosoma 53:107-117 (1975) [Exhibit 14] disclose the attachment of biotin to *Drosophila* rRNA via a cytochrome c bridge.

Politz et al., Biochemistry 20:372-378 (1981) [Exhibit 15] disclose the cross-linking of RNA to protein in *Escherichia coli* 30S ribosomal subunits using a heterobifunctional cross-linking reagent.

Cramer et al., Chemische Berichte 92:384-391 (1959) [Exhibit 16] disclose the attachment of polynucleotide sequences to polysaccharides in which 20 units of the latter was described as preferred.

In Paragraph 10A, Dr. Engelhardt states his opinion that the portions in the specification cited in his Declaration adequately describe the presently claimed invention, including the Sig component set forth in the claims. His opinion extends to all of those embodiments cited by the Examiner in the June 20, 1996 Office

(page 3, first paragraph) where:

- Sig is a moiety containing at least three carbon atoms (claims 280, 312, 340, 376, 408 and 436);
- Sig includes a glycosidic linkage moiety (claims 287, 319, 347, 380, 412 and 443);
- Sig is selected from biotin and iminobiotin (claims 288, 321, 349, 381, 413 and 449);
- Sig comprises ferritin (claims 289, 322, 350, 382 and 414); and
- Sig is selected from an antigen, a hapten and an antibody (claims 288, 321, 349, 381, 413 and 449).

In Paragraph 10A, Dr. Engelhardt expresses his further opinion that the specification reasonably conveys the description that Sig may be any of the foregoing when attached to the phosphate moiety in the presently claimed nucleotides and other composition claims comprising the nucleotides because of numerous instances (nine in all!) where Sig is described as being attached to the phosphate moiety P, the sugar moiety S and/or the base moiety B. The language in original claim 143 is highly significant in Dr. Engelhardt's opinion because it specifically recites "said nucleotide having covalently attached to the P or S or B moiety a chemical moiety Sig . . ." As he explains, the fact that dependent claims for the various embodiments of Sig were not included with the originally filed claims directed to the phosphate modified nucleotides (claim 141) does not in any way detract from his conviction and opinion that the support and description for such claims would have been clearly and reasonably conveyed by reading the specification, as described in the portions cited above. In Dr. Engelhardt's opinion, it is very clear that the specification discloses that the embodiments of Sig are to be applied - without limitation - in the disruptive and semi-disruptive positions of all three moieties recited in the independent claims, i.e., the base, sugar and phosphate moieties.

Elaborating further in Paragraph 10B, Dr. Engelhardt notes that the claimed products encompassing the various embodiments for Sig being attached to the phosphate moiety are clearly supported by the specification because all such embodiments for Sig are described as functionally equivalent for purposes of the present invention which is directed to disruptive and semi-disruptive modifications of nucleotides involving the phosphate, sugar and base moieties. He points out

that the fact that one description of the phosphate-modified nucleotides is found in a paragraph that opens with "By way of summary" is of no import for at least three substantial reasons:

- **First**, the nine separate instances in the specification where the attachment of Sig to any or all of the phosphate, sugar and base moieties is disclosed.

- **Second**, the paragraph beginning with "By way of summary" itself specifically discloses that "[t]he nucleotides are then modified in accordance with the practices of this invention by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig.

- **Third**, in the subsequent three paragraphs (page 96, last paragraph, through page 98, first paragraph) that describe embodiments for Sig, at least two instances occur where Sig is described as being attached to the phosphate, sugar or base moieties of the nucleotide.

According to Dr. Engelhardt, one could only conclude from reading the three paragraphs describing the embodiments for Sig that each are applicable to not only the base moiety, but the phosphate moiety (and the sugar moiety) as well. All of the foregoing reasons supports Dr. Engelhardt's conclusion that the specification reasonably conveys that the coinventors were in possession of the claimed subject matter.

Lastly on the description issue, Dr. Engelhardt points out that numerous reactions were known in the art for modifying the phosphate moiety of a nucleotide. It is his opinion that the specification as filed originally in 1982 reasonably conveys that the coinventors were in possession of the subject matter now being claimed and that that impression would not and does not require recitation of the litany of "phosphate-Sig" reactions indicated by the Examiner in the June 25, 1996 and June 20, 1997 Office Actions. As Dr. Engelhardt points out, phosphate-Sig reactions were known in the art and an explicit description of such known reactions would not have been necessary to convey the impression that the coinventors were in possession of the subject matter now being claimed.

In closing, it should not be overlooked or ignored that the disclosure need not contain a word-for-word description of the invention being claimed in order to satisfy the written description requirement of §112, first paragraph. In other words, a "literal basis" or "explicit description" of the claimed subject matter in the

disclosure is not the statutory standard for adequate. All that is required under the law is that the application reasonably convey the claimed subject matter. See, for example, Ex parte Parks, 30 U.S.P.A. 2d 1234 (B.P.A.I. 1994).

In view of Dr. Engelhardt's Declaration and its information and attached exhibits, it is respectfully submitted that an adequate description in the disclosure for the presently claimed subject matter has been clearly established. Reconsideration and withdrawal of this objection and rejection is believed to be in order.

The Objection and Rejection Under 37 C.F.R. §112, First Paragraph

The specification stands objected to under 35 U.S.C. §112, first paragraph, for allegedly failing to adequately teach how to make and/or use the invention, i.e. failing to provide an enabling disclosure. Claims 204-224 and 227-262 were also rejected under 35 U.S.C. §112, first paragraph, for the reasons set forth in the objection to the specification.

In the Office Action (page 3), the Examiner stated:

> Applicant argues that Example V, citing Halloran et al., describes labeling of the phosphate moiety with "Sig." This argument is not persuasive for two reasons.
>
> First, essential subject matter cannot be incorporated by reference to a research article. Second, the claims are not limited to a carbodiimide mediated linking of proteins to nucleotides but read generically on any "Sig" linked to the phosphate moiety by any method. Thus, the scope of the described subject matter is very different from the scope of the claimed subject matter. This difference in scope is reflected in the response filed 12/20/96, page 6, first paragraph: "[I]t is evident that at least one means of coupling nucleotide and oligonucleotides to labels through the phosphate moiety was available . . . " Since support for the subject matter of the same scope was not found, nor was it pointed out, the rejection under 35 U.S.C. §112, first paragraph, description requirement is MAINTAINED.
>
> Applicant argues that the claims of U.S. Pat. 5,260,433 is evidence of descriptive support and enablement for the present claims. This argument is not persuasive. Each case is argued on its own merits. Any arguments made in other cases must be made of record in this case in order to be considered.

The objection and rejection for nonenablement are respectfully traversed.

In response, Applicants refer once again to the Declaration of Dr. Dean L. Engelhardt (Exhibit A), a named coinventor and a senior vice president of the instant assignee. As indicated earlier in the description issue, Dr. Engelhardt's knowledge and experience qualify him to make his statement as a person skilled in the art. In his Declaration (Paragraph 11A), Dr. Engelhardt states it is his opinion that the specification provides an enabling disclosure for all of the pending claims in this application. Dr. Engelhardt points to Example V in the specification (page 57) as providing a means for labeling the oxygen or the phosphorus of a nucleotide, as well as to the chemistry and reactions for attaching substituents to the oxygen or phosphorus atoms in a nucleotidyl phosphate or phosphoric acid moiety that were already known in the art at the time the initial application was filed in 1982. In support of his opinion, Dr. Engelhardt refers to the publications cited with respect to the description:

Chemistry/Reaction	Citation	Description
oxygen	Goody et al. (1971) [Exhibit 2]	diphenyl addition to oxygen of nucleoside di- & triphosphates
oxygen	Eckstein (1975) [Exhibit 3]	methyl, aminoethyl, acetylaminoethyl & phenyl added to GDP & GTP
oxygen	Armstrong et al. (1976) [Exhibit 4]	methyl & phenyl attached to ATP & UTP analogs
phosphorus	Miller et al. (1979) [Exhibit 5]	methylene addition to prepare dideoxyribo-nucleoside methylphos-phonates
phosphorus	Miller et al. (1981) [Exhibit 6]	methylene addition to prepare dideoxyribo-nucleoside methylphos-phonates
phosphorus	Beaucage (1981) [Exhibit 7]	preparation of deoxynucleoside phosphoramidites
phosphorus	Miyoshi et al. (1980) [Exhibit 8]	oligonucleotide synthesis using phosphotriesters
phosphorus	Gait et al. (1980) [Exhibit 9]	oligodeoxynucleotide preparation using phosphotriesters

phosphorus	Duckworth et al. (1981) [Exhibit 10]	heptadecadeoxyribonucleotide preparation using phosphotriesters
phosphorus	Ohtsuka et al. (1982) [Exhibit 11]	dodecadeoxynucleotide preparation using phosphotriesters
phosphorus	Gough et al. (1981) [Exhibit 12]	oligodeoxyribonucleotide preparation using phosphotriesters
coupling	Halloran et al. (1966) [Exhibit 1]	coupling biotinylated poly-L-lysine to mono- and oligonucleotides
coupling	Brutlag et al. (1969) [Exhibit 13]	DNA to histone
coupling	Manning et al. (1975) [Exhibit 14]	biotin to rRNA using cytochrome c bridge
coupling	Politz et al. (1981) [Exhibit 15]	RNA to ribosomal protein
coupling	Cramer (1959) [Exhibit 16]	polynucleotide sequences to polysaccharides

In Paragraph 11B, Dr. Engelhardt states his opinion that the subject matter now being claimed in this application, claims 278-453, could have been practiced in 1982 - with minimal experimentation and not with excessive experimentation - from a reading of the specification, particularly Example V, and further in light of the chemistry and reactions known at that time. The known chemistry and reactions are illustrated by the scientific publications cited in Dr. Engelhardt's Declaration above (Exhibits 1-16).

In view of Dr. Engelhardt's Declaration with respect to the enablement issue, it is submitted that the grounds for the objection and rejection have been altogether obviated. Applicants respectfully request, therefore, reconsideration and withdrawal of the objection and rejection for lack of enablement.

The Rejection Under 35 U.S.C. §102(b)

Claims 204-206, 217, 234-240, 251, 259, 261 and 264 stand rejected under 35 U.S.C. §102(b) as being anticipated by Mackey et al., Biochemistry 16(20):4478-4482, 1977 (Mackey) for reasons of record.

In the Office Action (page 4), the Examiner stated:

> Applicant argues that a ^{32}P isotope is not a chemical modification or a chemical labeling. This argument is not persuasive. Radioactive isotopes have been the most common label used throughout analytical science. Radioactive labels are among the most sensitive and the most reliable. Also, claim 217 specifically recites "wherein Sig comprises a radioactive component."
>
> Applicant also argues that "the radioactive atom [is] carried by a chelator" and thus not attached to the phosphate moiety of the oligonucleotide. This argument is not persuasive. The radioactive isotope ^{32}P is attached directly to the phosphate moiety. For example, "gamma" labeled ^{32}P ATP has the radioactive isotope in the third phosphate which is attached to the first and second phosphate moiety of ATP.

The anticipation rejection is respectfully traversed.

As noted in the opening remarks of this Amendment, Applicants have added new claims which in every instance recites that the Sig moiety is capable of non-radioactive detection. This recitation is seen in each of new independent claims 278, 310, 338, 373, 405 and 433, which are directed to the claimed compositions in accordance with this invention, including a nucleotide, an oligo- or polynucleotide, and a composition comprising a polymeric compound. In each instance, Sig is defined as a moiety capable of non-radioactive detection. Furthermore, all other recitations in the claims previously directed to radioactive embodiments have been deleted. Thus, in none of the Markush claims related to Sig or the detectable moiety is a radioactive means for detection recited therein.

In light of the presentation of new claims 278-453, none of which is directed to radioactive labels, radioactive isotopes or radioactive detection, the anticipation rejection has been rendered moot.

<u>The First Obviousness Rejection Under 35 U.S.C. §103</u>

Claims 215, 216, 221-224, 231, 233, 249, 250, 255-258 and 265-277 stand rejected under 35 U.S.C. ɜ103 for being unpatentable over Gohlke et al., U.S. Patent No. 4,378,458, filed on March 30, 1981 (Gohlke) for reasons of record.

In the June 25, 1996 Office Action (page 5), the Examiner stated:

> Applicant argues "How can the invention not be enabled by the specification in view of the prior art and at the same time be obvious in view of the prior art."
>
> Standing alone, this argument against a double standard is a plausible proposition. However, when considered in light of the specific provisions of 35 U.S.C. §103, and §112 as it has been interpreted, it is seen to be untenable. 35 U.S.C. §112 provides that, in return for the grant of monopoly, the specification must enable one skilled in the art to "make and use" the invention without "undue experimentation" whereas 35 U.S.C. §103 makes no such requirement. Thus, a teaching of how to use a compound can be entirely adequate to render a claim obvious but, at the same time, entirely inadequate to support the allowance of such a claim.
>
> Applicant argues that Gohlke cannot be used generally or universally for labeling polynucleotides because DNA will not dissolve in the disclosed solvent.
>
> This argument is not persuasive. The teaching of the prior art need only suggest an embodiment within the claimed invention. The prior art teaching need not be as broad as the claimed invention. Also, Gohlke et al. specifically discloses that the products of the labeling reactions are functional (see for example Gohlke col. 6, lines 1-7). Furthermore, the claims are not limited to a method of labeling where DNA is dissolved in a particular solvent. The claims read on products made by the methods of Gohlke where the nucleotides are first labeled and then incorporated into the DNA molecule.

In the prior June 20, 1996 Office Action, the Examiner had stated:

> Gohlke discloses, for example, col 3, lines 3-22, the use of detection assays using labels such as fluorescent compounds, chemiluminescent compounds and enzymes like ß-galactosidase and in col. 2, lines 32 and 35, antibodies.
>
> The claims differ from Golke in the explicit recitation of the attachment at the phosphate moiety. However, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to attach these labels at the phosphate moiety because, as explicitly stated in Gohlke, the resulting product could be used to monitor the activity of ribonucleases (Gohlke col. 3, lines 22-35). Also, the phosphate moiety is where the other Gohlke detectable labels are attache (see Gohlke, col. 6, lines 25-51).

The obviousness rejection is respectfully traversed.

It is respectfully submitted that the rejection for *prima facie* obviousness cannot be maintained for several very significant reasons. **First**, no basis exists in

the art for modifying the cited document, Gohlke et al., as suggested by the Examiner. **Second**, Gohlke's substrates are disclosed for use in immunoassays, and in particular, for monitoring catalytic or enzymatic activity of an enzyme. As such, the art of immunoassays and enzymatic activity represented by Gohlke et al. is nonanalogous to the present invention that concerns nucleic acid modifications and technology. **Third**, all of the instant claim limitations must be considered and evaluated vis-a-vis Gohlke et al. **Fourth**, Gohlke et al. does not teach in any way the source of the problem overcome by the present invention, namely, the chemical modification of a nucleotide with a detectable moiety Sig in the so-called non-Ward disruptive and non-disruptive positions, i.e., the phosphate moiety, such that the chemically modified nucleotide can be incorporated into an oligo- or polynucleotide (or other polymer-containing composition) and, moreover, be capable of non-radioactive detection.

No basis exists for attempting the proposed modification to Gohlke's substrates

With regard to the first point, the Examiner has taken the position that Gohlke's stated goal of monitoring ribonuclease activity apparently provided sufficient motivation for modifying any phosphate in a nucleotide (regardless of its placement in the nucleotide) because "the resulting product could be used to monitor the activity of ribonucleases (Gohlke col. 3, lines 22-35). The Examiner has also pointed out that it is the phosphate moiety that bears Gohlke detectable labels, pointing to the disclosure in col. 6, lines 25-51.

As noted above, claim 278 and 373, the independent claim directed to Applicants' nucleotide, recite that PM is a di-phosphate or a tri-phosphate moiety. In and of itself, the foregoing amendment should be a sufficient basis for removing this obviousness rejection based upon Gohlke et al., that document only showing a single monophosphate.

The mere fact that Gohlke intends to monitor ribonuclease activity is insufficient motivation to attempt the modification suggested by the Examiner. In fact, a reading of the cited document makes it clear that Gohlke et al. actually teach away from gross modifications such as that suggested in the rejection. What Gohlke et al disclose in column 3, lines 22-25 is that "[r]ibonucleases are

phosphodiesterases which specifically catalyze the hydrolysis of 3'-internucleotide phosphate ester bonds of ribonucleic acids." In later describing substrates previously disclosed in the art for monitoring ribonuclease activity as a prelude to describing their own substrates, Gohlke et al. refer to Crook's mononucleotide substrate, cytidine 2',3'-phosphate (column 4, lines 16-30); mononucleotide-3'-phosphodiesters disclosed by the Zan-Kowalczewska groups (column 4, lines 31-52); and 3'-uridylic acid phosphodiesters of 1-naphthol, 5-hydroxynaphthol and 4-methoxyphenol disclosed by Rubsamen. And in every instance of describing their own single molecule substrates, Gohlke et al. are confined to the 3' position of the ribose. See, for example, column 6, lines 23-35; column 10, lines 1-11; reaction scheme in columns 11 and 12; and Examples I-XIII. In every instance, the phosphate in Gohlke's substrates is on the 3' ribose position.

Even more telling are Gohlke's self-imposed restraints for their substrates, the restraints being disclosed in column 6, line 52, and continuing through column 7, line 66. After describing their substrate for the first time, Gohlke et al. proceed to elaborate on "certain steric constraints which must be met in order to provide a substrate suitable for monitoring the catalytic activity of ribonuclease A-induced hydrolysis" (lines 52-55), among which follow:

- the trans, cis orientation of the base B and substituents at positions 1' and 2',3', respectively, appear to have rigid structural constraints to provide a suitable substrate (column 6, lines 55-58)

- substituents at the 4' position, that is, CH_2OR' may apparently have a configuration where the CH_2OR' group is cis to both the 2' and 3' functional groups (column 6, lines 59-61)

- base B assists in some fashion in the enzyme- or catalytic-induced hydrolysis of the phosphate ester at the 3'-position . . . by . . . helping lock the substrate into an appropriate position in relation to the enzyme for hydrolysis . . . . and [f]urther . . . assist in the proton transfer involved in the hydrolysis (column 6, line 65, through column 7, line 3)

- from a functional standpoint, the selection of the base should take into account the following factors in addition to, of course, its effect on product stability: (1) any modulation (increase or decrease) of enzymatic activity, (2) the difficulty of synthesis, (3) the effect on endogenous enzymatic activity and (4) the solubility in aqueous or other mediums of interest should not be adversely affected to any significant extent. (column 7, lines 4-11)

- Other factors . . . possible effects on hydrolysis and non-specific medium induced hydrolysis. (column 7, lines 11-13).

The above-quoted portions from Gohlke et al. teach away from the modification that would have been required to reach the instant invention.

Taking all of these matters into account, including the substrates in the prior art, Gohlke's own monophosphate substrates, the steric constraints and the fact that Gohlke's art concerns the pernickety enzyme-substrate interactions involving ribonucleases, the ordinarily skilled person could only conclude that it would be better to leave things well enough alone - and not to modify Gohlke's substrates in order to reach the instantly claimed modified di-phosphate and tri-phosphate nucleotides and other compositions.

Gohlke's immunoassays and enzymatic monitoring represents nonanalogous art to the present invention that concerns nucleic acid technology.

It goes without saying that although one of ordinary skill in the art is presumed to be aware of all prior art in the field to which the invention under examination pertains, such a person is not presumed to be aware of nonanalogous prior art outside that field. In the situation at hand, Gohlke et al. worked in the field of immunoassays and more particularly, the detection and measurement of catalytic activity from an enzyme or polypeptide pair, specifically ribonuclease activity. Gohlke's inquiries were confined to protein interactions, most notably, the binding interactions that occur between an enzyme, i.e., ribonuclease, and a suitable substrate. Such binding interactions largely involve three-dimensional recognition. In other words, if the substrate fits the enzyme's three-dimensional informational requirements, it's a fit and the enzymatic reaction will proceed.

In sharp contrast to Gohlke et al., the present invention concerns nucleic acid technology, and in particular, the disruptive and semi-disruptive modification of nucleic acids for, among other purposes, detection of nucleic acid analytes. The geometry and information of nucleic acids, particularly when it comes to hybridization, is two-dimensional. That is, a nucleic acid sequence hybridizes to its counterpart along an axis. There are tertiary and quaternary instances of nucleic acid structure, but such fall outside the parameters for complementary nucleic acid

hybridization. The person of ordinary skill in the art would not look to the field of immunoassays and enzyme-substrate interactions for guidance into modifying nucleic acids for detection purposes, for example. The three-dimensional universe of proteins and enzymes simply does not coincide with the two-dimensional information world of nucleic acids. Thus, modifications to nucleic acids would not be obvious in light of modifications to proteins or enzymes and their substrates.

All claim limitations must be considered.

In evaluating any claim for the purpose of determining obviousness or nonobviousness, all limitations of the claim must be evaluated. In the instant claims, the phosphate moiety is clearly and unambiguously defined. In the case of the nucleotide claims, 278 et seq., and 373 et seq., PM is defined as a di-phosphate or a tri-phosphate moiety and the nucleotide is further defined as being capable of incorporation into an oligo- or polynucleotide. As indicated earlier, Gohlke et al. only disclose a monophosphate which is not shown to be capable of incorporation into an oligo- or polynucleotide, nor would it be expected to be so because the disclosed monophosphate serves as a substrate for an enzyme - and a ribonuclease at that.

Moreover, Gohlke et al. does not disclose or suggest an oligo- or polynucleotide, or a composition comprising a polymeric compound, in which their disclosed substrate is first, incorporated, and second, capable of producing a detectable non-radioactive signal. In contrast to Gohlke et al., Applicants disclose and claim an oligo- or polynucleotide, as well as a composition comprising a polymeric compound, in all cases of which at least one nucleotide is attached in which the phosphate moiety bears a Sig moiety capable of non-radioactive detection.

Thus, neither Gohlke's disclosure nor the modification proposed in the June 20, 1996 Office Action reaches the present invention which is patentably distinguishable on at least three grounds. First, the instantly claimed nucleotide, claims 278 et seq. and claims 373 et seq., are both directed to the di-phosphate and the tri-phosphate. Second, Applicants' nucleotides are capable of being incorporated into an oligo- or polynucleotide. Third, Applicants disclose and claim

compositions in the form of an oligo- or polynucleotide, and a polymeric compound, in which a phosphate modified nucleotide bearing Sig moiety capable of non-radioactive detection has been attached thereto. Gohlke et al. simply do not disclose or suggest the instantly claimed compositions.

Gohlke et al. does not teach the source of the problem solved by the present invention, namely, disruptive labelling of nucleic acids for detection and other purposes.

Because as explained above, Gohlke et al. is concerned with immunoassays and monitoring enzymatic or catalytic activity of ribonucleases, it is understandable that Gohlke is silent on the subject of the problem that was solved by the present invention. As noted in Dr. Dean L. Engelhardt's Declaration submitted in response to the description and enablement issues, the present invention embarked on a path far removed from the leading nucleic acid labeling technology of the time, the Ward technology which was directed to non-disruptive base modifications and has since become the industry standard. By extending into the realm of disruptive nucleic acid modifications in contradistinction to Ward's essential probe criteria for placement of ring substituents in the base, Applicants solved a problem that could not have even been contemplated, let alone addressed or solved by Gohlke et al.

In view of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the this obviousness rejection.

The Second Obviousness Rejection Under 35 U.S.C. §103

Claims 207-214, 219, 220, 227-230, 232, 241-248, 253, 254, 260 and 262 stand rejected under 35 U.S.C. §103 for being unpatentable over Gohlke in view of Sodja et al., Nucleic Acids Res, 5(2):385-401, 1978 (Sodja) for reasons of record.

In the prior June 20, 196 Office Action (page 8), the Examiner stated:

The teachings of Gohlke are explained above. Sodja teaches on page 386 the attachment, to the free 3' OH end of RNA, an avidin-ferritin label using the lysine groups of the polypeptide cytochrome-c.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use the labels of Sodja in the methods of Gohlke for the expected benefit of using electron microscopic detection of the bound label.

The second obviousness rejection is respectfully traversed.

As noted above in the first obviousness rejection, the present invention is distinguishable from Gohlke's disclosure on several fronts, not the least of which are the latter's substrate being limited to the monophosphate, and not to the diphosphate or triphosphate, and other compositions, including an oligo- or polynucleotide, as set forth in the instant claims. In addition, Gohlke et al. teach away from the modification necessary to reach the instant invention, particularly in view of the fact that severe constraints are placed on their substrates combined with their field of investigation, enzyme-substrate interactions, an altogether nonanalogous field to the present invention. The addition of Sodja and Davidson does not in any way cure the deficiencies in Gohlke et al.

Sodja and Davidson were concerned with gene mapping and gene enrichment. To do so, they performed a modification to RNA that was so severe that it destroyed the ribose sugar, in effect, destroying the nucleotide as a chemical entity. As disclosed on pages 386 and 387 in Sodja and Davidson's paper, their basic reaction scheme is as follows:

1) Oxidation of free 2', 3' OH ends of RNA to the dialdehyde with periodate.
2) Schiff base formation of the terminal dialdehyde with the polyamine, cytochrome-c, at relatively low ionic strength, and stabilization of the compound against dissociation and/or ß elimination by BH_4^- reduction.
3) Purification of RNA-cytochrome-c from free RNA and free cytochrome-c by sequential chromatography on carboxymethyl cellulose (CMC) and on hydroxyapatite (HAP).
4) Covalent attachment of several biotin molecules to lysine NH_2 groups of the cytochrome-c by acylation with the N-hydroxy succinimide (NHS) ester of the carboxylic acid biotin.
5) Hybridization of the RNA-cc-biotin to DNA.
6) Labeling with avidin-ferritin or avidin-spheres.
7) Gene mapping by electron microscopy or gene enrichment by banding in CaCl.

By oxidizing the 2' and 3' OH ends of RNA with periodate to form the dialdehyde (step 1 above), Sodja and Davidson broke open the ribose ring; thus destroying the integrity of the sugar and the nucleotide as well. Such a destructive procedure would never lead to the present invention. Thus, when Sodja and Davidson fomed a Schiff base by reacting the terminal dialdehyde with the polyamine, cytochrome c (step 2 above), they were not working with a nucleotide at that point but some other chemical entity or molecule. Moreover, in that reaction, cytochrome c was not reacting with a phosphate moiety, as required by the present invention. Furthermore, in labeling their purified RNA-cytochrome c with biotin (step 4 above), Sodja and Davidson attached the biotin to the lysine NH_2 groups - not to a phosphate moiety. Thus, when labeling with avidin-ferritin or avidin-spheres (step 6 above), Sodja and Davidson were not remotely connected to the invention at hand.

Beyond that, it is difficult to reconcile Gohlke et al. with Sodja and Davidson. The former eschews gross modifications because of concern over the structural integrity of enzyme substrates. The latter embraces any modification no matter how destructive - as long as it will lead to an identifiable marker in gene mapping or gene enrichment.

In view of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of this obviousness rejection.

<u>The Third Obviousness Rejection Under 35 U.S.C. §103</u>

Claims 218 and 252 stand rejected under 35 U.S.C. ɜ103 as being unpatentable over Mackey in view of Roychoundhury et al.; Nucleic Acids Res, 3(1):101-16, Jan 1976 (Roychoundhury) for reasons of record.

In the Office Action (page 7), the Examiner stated that "Applicant argues the remaining rejections for the reasons given above. These arguments are not persuasive for the reasons given above."

In the prior June 20, 1996 Office Action (pages 8-9), the Examiner stated:

Roychoundhury et al. teaches the labeling of nucleotides with cobalt (see for example Roychoudhury, Abstract).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use a cobalt label, in addition to the ^{32}P label, for the expected benefit of measuring an additional radioactive decay product in multiple labeling experiments. The radioactive cobalt decay product has an energy level very different from that of the ^{32}P decay product and can be measured independently of the ^{32}P decay product in experiments where ^{32}P is being used to follow another species within a reaction mix.

This obviousness rejection is respectfully traversed.

In light of the cancellation of claims 218 and 252, the subject matter of which is no longer present in the new claims, the grounds for this rejection have been rendered moot. Withdrawal of the rejection is respectfully requested.

* * * * * * *

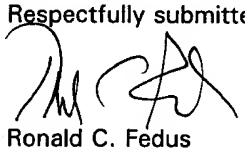
SUMMARY AND CONCLUSIONS

Claims 204-224 and 227-277 have been canceled and claims 278-453 have been added in their place for further examination.

The fee for the newly added claims 278-453 is $2,804, based upon the presentation of 104 additional claims above the 72 previously paid for claims, and the three additional independent claims above the three previously paid for independent claims. This response is being accompanied by a Request for an Extension of Time (two months) and authorization for the large entity fee therefor. In addition, a Request Under 37 C.F.R. §1.129(a) for Withdrawal of the Finality of the June 25, 1997 Office Action is also being made, again with authorization for the appropriate large entity fee therefor. In the event that any other fee is due for this response or any of the other concurrent filings, authorization is hereby given to charge the amount of any such fee to Deposit Account No. 05-1135, or to credit any overpayment thereto.

If it would be helpful to expediting prosecution of this application, the undersigned may be reached by telephone during business hours at 212-583-0100, or by facsimile at 212-583-0150.

Respectfully submitted,



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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: Dean L. Engelhardt et al.)
Serial No. 08/479,997) Group Art Unit: 1809
Filed: June 7, 1995) Examiner: Scott Houtteman
Title: A PHOSPHATE MOIETY LABELED NUCLEOTIDE, AND AN OLIGO- OR POLYNUCLEOTIDE, AND OTHER COMPOSITIONS COMPRISING SUCH PHOSPHATE MOIETY LABELED NUCLEOTIDES)

527 Madison Avenue, 9th Floor
New York, New York 10022

Honorable Commissioner of Patents and Trademarks
The United States Patent and Trademark Office
Washington, D.C. 20231

DECLARATION OF DR. DEAN L. ENGELHARDT IN SUPPORT OF ADEQUATE DESCRIPTION AND ENABLEMENT

I, Dean L. Engelhardt, hereby declare as follows:

1. I am the Dean L. Engelhardt who is named as an applicant on the above-identified application. I am a co-inventor of the subject matter claimed in this application. Furthermore, I am familiar with the contents of this application.

2. I am currently employed by Enzo Biochem, Inc., 527 Madison Avenue, New York, New York 10022 as Senior Vice President, having held that position since 1988. Prior to my employment at Enzo Biochem, Inc., I was Associate Professor of Microbiology at Columbia University College of Physicians and Surgeons, New York City, having earlier obtained my doctorate from Rockefeller University in New York City.

3. In addition to my position as Senior Vice President of Enzo Biochem, Inc., I have also served as Director of Research in which capacity I have overseen scientific research activities for the company and its subsidiaries. I also continue to oversee various research projects. Among my responsibilities at Enzo Biochem, Inc. have been the development of new nucleic acid technology and hybridization formats, including new diagnostic and therapeutic approaches and agents based upon nucleic acid technology.

4. I understand that the presently pending claims in this application are directed to a nucleotide in which a moiety Sig is covalently attached to the phosphate moiety (a di-phosphate or tri-phosphate) directly or via a chemical linkage. Sig is capable of non-radioactive detection when attached to the phosphate or when the nucleotide is incorporated into an oligo- or polynucleotide or other composition. I further understand that other presently pending claims are directed to an oligo- or polynucleotide and to other compositions including those comprising a polymeric compound - all of which comprise at least one nucleotide in which a moiety Sig capable of non-radioactive detection is attached to the phosphate moiety thereof directly or via a chemical linkage.

5. In further detail, I understand that the presently pending claims are directed to the just-described nucleotide (278-301, 308-309, 373-394 and 401-404), the oligo- or polynucleotide (310-337 and 405-432), and other compositions (303-307, 338-372 and 433-453) comprising phosphate-modified nucleotides in which a moiety Sig capable of non-radioactive detection is attached thereto directly or via a chemical linkage.

A. Claim 278 is independent and defines the nucleotide as having the formula

Sig - PM - SM - BASE

wherein PM is selected from a di-phosphate or a tri-phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from a pyrimidine, a purine and a deazapurine, or analog thereof. PM is attached to SM and BASE is attached to SM. Sig is covalently attached to PM directly or via a chemical linkage and it represents a moiety capable of non-radioactive detection when attached to PM. Furthermore, the claimed nucleotide is defined as being capable of incorporation into an oligo- or polynucleotide. Other embodiments of the aforementioned nucleotide include those defining the self-signaling or self-indicating or self-detecting nature of Sig (claim 279); the Sig moiety comprising at least three carbon atoms (claim 280); the

covalent attachment or chemical linkage of Sig to PM (claims 281-287); specific members of Sig (claim 288-301); and the nucleotide comprising a deoxyribonucleotide (claim 308) and a ribonucleotide (claim 309).

B. I also understand that the presently pending claims define an oligo- or polynucleotide comprising at least one such phosphate-modified nucleotide, the oligo- or polynucleotide being terminally ligated or attached to a polypeptide (claim 302). The claims also include other compositions comprising an oligo- or polynucleotide including at least one such phosphate-modified nucleotide and a polypeptide capable of forming a complex with Sig and a moiety which can be detected when the complex is formed (claims 303-307). My understanding of the present claims extend to the oligo- or polynucleotide of which claim 310 is independent and arguably the broadest. Claim 310 defines the oligo- or polynucleotide as comprising at least one nucleotide having the formula

Sig - PM - SM - BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from a pyrimidine, a purine and a deazapurine, or analog thereof. PM is attached to SM and BASE is attached to SM. Sig is covalently attached to PM directly or via a chemical linkage and represents a moiety capable of non-radioactive detection when attached to PM or when the nucleotide is incorporated into the oligo- or polynucleotide. Other dependent claims directed to this oligo- or polynucleotide include embodiments of the self-signaling or self-indicating or self-detecting nature of Sig (claim 311); Sig as comprising at least three carbon atoms (claim 312); the covalent attachment or chemical linkage of Sig to PM (claim 313-320); specific members of Sig (claims 321-334); the attachment of Sig to a terminal nucleotide (claims 335-337); and the nucleotide comprising a deoxyribonucleotide (claim 427) and a ribonucleotide (claim 428).

C. My understanding of the presently pending claims also extend to the composition comprising a polymeric compound having attached directly or indirectly thereto at least one nucleotide having the formula:

Sig - PM - SM - BASE

wherein PM is a phosphate moiety, SM is a sugar moiety and BASE is a moiety selected from a pyrimidine, purine and a deazapurine, or analog thereof. PM is attached to SM, BASE is attached to SM, and Sig is covalently attached to PM directly or via a chemical linkage and it represents a moiety capable of non-

radioactive detection when attached to PM and when the nucleotide is incorporated into such composition. Claim 338 is independent and is arguably the broadest such composition. Other dependent claims are directed to embodiments describing the self-signaling or self-indicating or self-detecting nature of Sig (claim 339); Sig as comprising at least three carbon atoms (claim 340); the covalent attachment or chemical linkage of Sig to PM (claims 341-348); specific members of Sig (claims 349-361); the composition and a complex-forming polypeptide (claims 363-368); and specific members of the polymeric component (claims 369-372).

D. I also understand that other claims are pending in which the aforementioned moieties (BASE, PM and Sig) are attached to a pentose ring sugar moiety (SM) given by a structural formula set forth in these other claims. The claims reciting such a structural formula include those that are directed to a phosphate-modified (a di-phosphate or tri-phosphate) nucleotide (claims 373-394 and 401-404); an oligo- or polynucleotide or other composition comprising at least one such nucleotide (claims 395-400); an oligo- or polynucleotide comprising at least one phosphate-modified nucleotide (claims 405-432); and a composition comprising a polymeric compound attached directly or indirectly to at least one such phosphate-modified nucleotide (claims 433-453).

6. I have read the two Office Actions dated June 20, 1996 and June 25, 1997 that were issued in connection with this application. I understand that in both Office Actions the specification of this application was objected to and the claims were rejected for lack of adequate description and enablement.

A. The Examiner's position on the description issue taken from the June 20, 1996 Office Action is as follows:

> Claims 207-224 and 227-262 are drawn to nucleotides having the "Sig" moiety attached to the phosphate moiety wherein the Sig moiety is limited to one of several molecular classes such as "at least three carbon atoms, a glycosidic linkage moiety, biotin, iminobiotin, ferritin, an antigen, a hapten, an antibody, etc.
>
> Support for these claims was pointed out in original claims 125, 41, 84, 126, 129, 127 and 128. However, these claims are drawn to nucleotides in which the "Sig" moiety is attached to the base.
>
> The only support that was found in the original disclosure was in a passage on pages 96-97 which begins "By way of summary." This passage defines "Sig" as binding to either base, sugar or phosphate and then defines "Sig" to include the particular products in

the newly presented claims. However, there is no explicit description of the various claimed products bound to the phosphate anywhere in the specification. In contract, the base-linked "Sig" moieties have numerous complex chemical reactions which are necessary to synthesis the various products. These reactions include various solvents, reactants and protecting groups which are necessary so that only the base was modified and not the reactive groups on the sugar or phosphates. Thus, an explicit description of the "phosphate-Sig" reactions would have been expected in order for a skilled artisan to have reasonably concluded that the original disclosure evidenced "possession" of the currently claimed invention.

Thus, in view of the phrase "by way of summary" and the absence of any "phosphate-Sig" reactions to summarize; and in view of the complex nature of these reactions, the skilled artisan would not have reasonably expected this specification to put the artisan in possession of the invention as now claimed.

Since support for these claims was not found where pointed out nor elsewhere in the specification, these claims are considered "new matter."

B. The Examiner's position on enablement as set forth in the June 20, 1996 Office Action is as follows:

Claims 204-224 and 227-262 are broadly drawn to nucleotides having various "Sig moieties" attached to the phosphate moiety.

The specification contains a sufficiently detailed disclosure, such as in Examples I-VII, to enable the construction of "sig-base" nucleotides, that is nucleotides in which the "Sig" moiety is linked to the base. It is noted that these reactions contain many specific solvents, reactants and protecting groups. This detailed disclosure enables one to obtain a reasonable product yield, a product of suitable stability for it's intended use in nucleic acid detection assays and a product reasonably free of unwanted side products in which the Sig moiety is attached at the wrong places on the nucleotide.

However, there is no analogous disclosure for the attachment of the "Sig-phosphate" nucleotides. The broadly claimed "Sig moieties" include a very diverse population of molecules, from simple inorganic compounds like radioactive cobalt to the complex organic molecules like enzymes. Accordingly, there are a vast number of possible chemical reaction schemes one could attempt. Without specific guidance or examples, the skilled artisan would expect that the vast majority of these reaction schemes would fail. Either the product yields would be low, the products would be too unstable or the products would be too hard to purify away from extraneous side products.

It is difficult to predict the behavior of a complex organic molecule with numerous functional groups: primary amines, carboxyl groups and alcohol groups. There is no way to establish, before the fact, which reaction conditions will result in high yields and stable products that can be purified from extraneous byproducts.

The level of skill is high in this field. Nevertheless, in view of the large scope of these claims, the lack of any guidance or specific

examples, the high degree of unpredictability, the complex nature of the invention which requires both inorganic and organic chemical syntheses; it would have required undue experimentation to enable a reasonable number of embodiments within the scope of these claims.

C. I also understand that the enablement issue was maintained by the Examiner in the most recent June 25, 1997 Office Action, the Examiner stating there:

> Applicant argues that Example V, citing Halloran et al., describes labeling of the phosphate moiety with "Sig." This argument is not persuasive for two reasons.
>
> First, essential subject matter cannot be incorporated by reference to a research article. Second, the claims are not limited to a carbodiimide mediated linking of proteins to nucleotides but read generally on any "Sig" linked to the phosphate moiety by any method. Thus, the scope of the described subject matter is very different from the scope of the claimed subject matter. This difference in scope is reflected in the response filed 12/20/96, page 6. first paragraph: "[I]t is evident that at least one means of coupling nucleotide and oligonucleotides to labels through the phosphate moiety was available . . ." Since support for the subject matter of the same scope was not found, nor was it pointed out, the rejection under 35 U.S.C. §112, first paragraph, description requirement is MAINTAINED.
>
> Applicant argues that the claims of US Pat. 5,260,433 is evidence of descriptive support and enablement for the present claims. This argument is not persuasive. Each case is argued on it's own merits. Any arguments made in other cases must be made of record in this case in order to be considered.

7. It is my opinion that the originally filed specification does indeed support the subject matter of the pending claims which are adequately described to the point that a skilled artisan would have reasonably concluded that the original disclosure evidenced possession of the invention currently being claimed. It is also my opinion that the specification provides a disclosure sufficiently enabling so that the skilled artisan, armed with the disclosure and knowledge in the art at the time the application was originally filed in 1982, would have been able to practice the claimed invention without excessive experimentation, or to put it in other words, to practice the invention with a minimum of experimentation. I am making this Declaration to substantiate both the support and adequate description in the specification for the claims and the enabling nature of the specification.

8. A. With respect to the support and description in the specification for the presently claimed invention, I offer the following remarks. In order to understand the basis of this invention, it would be helpful to describe briefly the state of

technology with respect to nucleic acid labeling and detection in the early 1980s. In 1981, Dr. David C. Ward and his group at Yale became textbook celebs for their discovery that nucleotides could be non-radioactively labeled in the so-called non-disruptive positions of the base without substantially interfering with the capability of the labeled nucleotide to be incorporated into an oligo- or polynucleotide, and without substantially interfering with the capability of the oligo- or polynucleotide to be detected by means of the labeled nucleotide that was incorporated. Prior to 1981, nucleic acids were conventionally labeled with radioactive isotopes, most notably ^{32}P. With Dr. Ward's discovery, the world turned en masse to non-radioactive labeling of nucleic acids, that discovery culminating in the issuance of several United States and foreign patents including the following: U.S. Patent Nos. 4,711,955; 5,328,824; 5,449,767; 5,476,928; and European Patent Nos. 0 063 879 B1 and 0 329 198 A2. The latter is an allowed application that has not yet been formally granted.

B. The principles or criteria behind the Ward discovery are exquisitely set forth in their patent specifications. In U.S. Patent No. 5,328,824, for example, the Ward "criteria" for base labeling nucleotides are described in columns 6 and 7 under the section titled "DETAILED DESCRIPTION OF THE INVENTION:'

> Several essential criteria must be satisfied in order for a modified nucleotide to be generally suitable as a substitute for a radioactively-labeled form of a naturally occurring nucleotide. First, the modified compound must contain a substituent or probe that is unique, i.e., not normally found associated with nucleotides or polynucleotides. Second, the probe must react specifically with chemical or biological reagents to provide a sensitive detection system. Third, the analogs must be relatively efficient substrates for commonly studied nucleic acid enzymes, since numerous practical applications require that the analog be enzymatically metabolized, e.g., the analogs must function as substrates for nucleic acid polymerases. For this purpose, probe moieties should not be placed on ring positions that sterically, or otherwise, interfere with the normal Watson-Crick hydrogen bonding potential of the bases. Otherwise, the substituents will yield compounds that are inactive as polymerase substrates. Substitution at ring positions that alter the normal "anti" nucleoside conformation also must be avoided since such conformational changes usually render nucleotide derivatives unacceptable as polymerase substrates. Normally, such considerations limit substitution positions to the 5-position of a pyrimidine and the 7-position of a purine or a 7-deazapurine.
>
> Fourth, the detection system should be capable of interacting with probe substituents incorporated into both single-stranded and double-stranded polynucleotides in order to be compatible with nucleic

acid hybridization methodologies. To satisfy this criterion, it is preferable that the probe moiety be attached to the purine or pyrimidine through a chemical linkage or "linker arm" so that it can readily interact with antibodies, other detector proteins, or chemical reagents.

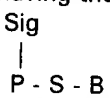
Fifth, the physical and biochemical properties of polynucleotides containing small numbers of probe substituents should not be significantly altered so that current procedures using radioactive hybridization probes need not be extensively modified. This criterion must be satisfied whether the probe is introduced by enzymatic or direct chemical means.

Finally, the linkage that attaches the probe moiety should withstand all experimental conditions to which normal nucleotides and polynucleotides are routinely subjected, e.g., extended hybridization times at elevated temperatures, phenol and organic solvent extraction, electrophoresis, etc.

All of these criteria are satisfied by the modified nucleotides described herein.

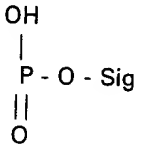
9. A. A short time after Ward's discovery, it was unexpectedly discovered by the instant inventors - all of whom were scientists at Enzo - that nucleic acid labeling and detection could be extended far beyond, but moreover, in total contradiction to Ward's discovery and criteria. Our subsequent and unexpected discovery that culminated in the filing of the first application in the family in 1982 flew headlong against Ward because the positions for labeling the nucleic acid now involved the so-called "disruptive" and "semi-disruptive" positions in the base. Moreover, the novel labeling and labeled compositions involved not only such positions in the base moiety, but the sugar and phosphate moieties as well. This discovery with respect to the phosphate moiety is set forth in several portions in the instant specification. In the specification, page 94, last paragraph, and continuing through page 95, first paragraph, the phosphate-modified nucleotides and compositions of the present invention are specifically disclosed but not for the first time:

Still further, nucleotides in accordance with the practices of this invention include the nucleotides having the formula,



wherein P is the phosphoric acid moiety, S the sugar moiety and B the base moiety. In these special nucleotides the P moiety is attached to the 3' and/or the 5' position of the S moiety when the nucleotide is deoxyribonucleotide and at the 2', 3' and/or 5' position when the nucleotide is a ribonucleotide. The base B is either a purine or a pyrimidine and the B moiety is attached from the N1 or the N9 position to the 1' position of the sugar moiety when said B moiety is a

pyrimidine or a purine, respectively. The Sig chemical moiety is covalently attached to the phosphoric acid P moiety via the chemical linkage



said Sig, when attached to said P moiety being capable of signalling itself or making itself self-detecting or its presence known and desirably the nucleotide is capable of being incorporated into a double-stranded or DNA, RNA or DNA-RNA hybrid.

Later on page 96, and continuing through the first paragraph on page 98, further description of the present invention is amply provided:

By way of summary, as indicated hereinabove with respect to the make-up of the various special nucleotides in accordance with this invention, the special nucleotides can be described as comprising a phosphoric acid moiety P, a sugar moiety S and a base moiety B, a purine or pyrimidine, which combination of P-S-B is well known with respect to and defines nucleotides, both deoxyribinucleotides and ribonucleotides. The nucleotides are then modified in accordance with the practices of this invention by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig. The chemical moiety Sig so attached to the nucleotide P-S-B is capable of rendering or making the resulting nucleotide, now comprising P-S-B with the Sig moiety being attached to one or more of the other moieties, self-detecting or signalling itself or capable of making its presence known per se, when incorporated into a polynucleotide, especially a double-stranded polynucleotide, such as double-stranded DNA, a double-stranded RNA or a double-stranded DNA-RNA hybrid. The Sig moiety desirably should not interfere with the capability of the nucleotide to form a double-stranded polynucleotide containing the special Sig-containing nucleotide in accordance with this invention and, when so incorporated therein, the Sig-containing nucleotide is capable of detection, localization or observation.

The Sig moiety employed in the make-up of the special nucleotides of this invention could comprise an enzyme or enzymic material, such as alkaline phosphatase, glucose oxidase, horseradish peroxidase or ribonuclease. The Sig moiety could also contain a fluorescing component, such as fluorescein or rhodamine or dansyl. If desired, the Sig moiety could include a magnetic component associated or attached thereto, such as a magnetic oxide or magnetic iron oxide, which would make the nucleotide or polynucleotide containing such a magnetic-containing Sig moiety detectable by magnetic means. The Sig moiety might also include an electron dense component, such as ferritin, so as to be available by observation. The Sig moiety could also include a radioactive isotope component, such as radioactive cobalt, making the resulting nucleotide observable by radiation detecting means. The Sig moiety could also include a hapten component or per se be capable of complexing with an antibody specific thereto. Most usefully, the Sig moiety is a polysaccharide or

oligosaccharide or monosaccharide, which is capable of complexing with or being attached to a sugar or polysacchardie binding protein, such as a lectin, e.g. Concanavilin A. The Sig component or moiety of the special nucleotides in accordance with this invention could also include a chemiluminescent component.

As indicated in accordance with the practices of this invention, the Sig component could comprise any chemical moiety which is attachable either directly or through a chemical linkage or linker arm to the nucleotide, such as the base B component therein, or the sugar S component therein, or the phosphoric acid P component thereof.

The Sig component of the nucleotides in accordance with this invention and the nucleotides and polynucleotides incorporating the nucleotides of this invention containing the Sig component are equivalent to and useful for the same purposes as the nucleotides described in the above-identified U.S. patent application Serial No. 255,223. More specifically, the chemical moiety A described in U.S. patent application Serial No. 255,223 is functionally the equivalent of the Sig component or chemical moiety of the special nucleotides of this invention. Accordingly, the Sig component or chemical moiety of nucleotides of this invention can be directly covalently attached to the P, S or B moieties or attached thereto via a chemical linkage or linkage arm as described in U.S. patent application Ser. No. 255,223, as indicated by the dotted line connecting B and A of the nucleotides of U.S. Serial No. 255,223. The various linker arms or linkages identified in U.S. Ser. No. 255,223 are applicable to and useful in the preparation of the special nucleotides of this invention.

Even further embodiments of the instant nucleotides and compositions are later described in the specification, on page 103, first full paragraph; and on page 103, last paragraph, continuing through page 106, first paragraph.

B. In all, there are no fewer than nine (9) instances where the Sig moiety component is described in the specification as being attached to the phosphate moiety P, the sugar moiety S and/or the base moiety B! These nine separate and distinct instances include the following:

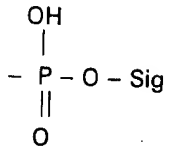
Specification	Description
page 90, last paragraph	. . . and a signalling chemical moiety Sig covalently attached thereto, either to the P, S or B moiety.
page 93, first paragraph	. . . include a chemical moiety Sig covalently attached to the P, S and/or B moieties.
page 96, first paragraph	. . . by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig.

page 98, first paragraph	. . . the Sig component or chemical moiety of nucleotides of this invention can be directly covalently attached to the P, S or B moieties or attached thereto via a chemical linkage or linkage arm . . .
page 103, first full paragraph	. . . and the signalling or self-detecting moiety, Sig, covalently attached to either the P, S or B moieties, as indicated hereinabove, . . .
page 104, first paragraph	. . . nucleotides in accordance with this invention containing the above-described components P, S, B and Sig, . . .
page 105, first paragraph	. . . the nucleotides of this invention include the P, S, B and Sig components wherein the Sig is covalently attached to either the P, S or B moieties
page 105, second paragraph	The moiety Sig attached to the special nucleotides of this invention containing the other moieties or components P, S, B provides a site per se for the attachment thereto, the Sig component, . . .
page 106, first paragraph	. . . the special P, S, B and Sig-containing nucleotides of this invention, . . .

C. In addition to those portions in the specification cited above, Example V describes a method for attaching biotin, one of the embodiments for Sig, to the phosphate moiety of a mononucleotide and an oligonucleotide that are coupled to a protein, poly-L-lysine. Using the procedure in Example V in the specification (page 57), the biotinylated poly-L-lysine is coupled to a terminal oxygen of the phosphate moiety or to a terminal phosphorus. These reaction schemes are set forth in Figure 1 on page 374 in Halloran and Parker, J. Immunol., 96:373 (1966) cited in Example V, page 57 in the specification (a copy of Halloran's publication also having been attached hereto as Exhibit 1).

D. In my opinion, the originally filed claims are a telling piece of evidence with respect to using any of the embodiments of Sig for either the base, sugar or phosphate moieties. Here, one need only look at original claim 143 that recites:

> A nucleotide having the general formula P–S–B wherein P is the phosphoric acid moiety, S the sugar or monosaccharide moiety and B the base moiety, said nucleotide having covalently attached to the P or S or B moiety a chemical moiety Sig, said Sig chemical moiety when attached to the P moiety is attached thereto via the chemical linkage,



and when Sig is attached to the S moiety, the S moiety is a ribose group, said chemical moiety Sig when attached to said P, S or B being capable of signalling itself or makes itself self-detecting or its presence known.

It is clear from the language of original claim 143 that Sig could be attached to the phosphate (or phosphoric acid), sugar and base moieties in accordance with this invention.

E. The chemical reactions by which substituents are attached to the oxygen or the phosporus atoms of a phosphate or phosphoric acid moiety in a nucleotide (or an oligo- or polynucleotide or other polymer such as a protein) were known in the art prior to the first filing of this application in 1982. Illustrative of the reactions and chemistry known in the art before 1982 are those summarized below.

Reactions involving the oxygen

Goody et al., JACS 93:6252-6257 (1971) [Exhibit 2] disclose a reaction for adding a diphenyl to the oxygen atoms of nucleoside di- and triphosphates. See, for example, Scheme I on page 6253.

Eckstein et al., Biochemistry 14:5225-5232 (1975) [Exhibit 3] disclose guanosine 5'-di- and triphosphate derivatives with modified terminal phosphates in which the following substituents are added to the terminal oxygen: methyl, aminoethyl, acetylaminoethyl and phenyl. See, for example, the discussion under "*Synthesis of Analogues*" beginning on page 5226, left column, and continuing through page 5228, right column. See also Figure 1 on page 5226.

Armstrong et al., European Journal of Biochemistry 70:33-38 (1976) [Exhibit 4] disclose ATP and UTP analogues modified in the phosphate moieties in which a methyl or a phenyl group is attached to a terminal oxygen. See structures I b) and I c) on page 33, right column.

Reactions involving the phosphorus

Miller et al., Biochemistry 18:5134-5143 (1979) [Exhibit 5] disclose a series of dideoxyribonucleoside methylphosphonate analogues in which a methylene group is contained in the internucleoside linkage. See, for example, the discussion under "*Preparation of Dinucleoside Methylphosphonates*" beginning on page 5136, right column, and continuing through page 5137.

Miller et al., Biochemistry 20:1874-1880 (1981) [Exhibit 6] disclose the preparation of oligodeoxyribonucleoside methylphosphonates. See, for example, the discussion under "*Preparation of Oligonucleoside Methylphosphonates*" beginning on page 1875, left column, and continuing through the first four lines of the right column. See also Table I at the top of page 1876.

Beaucage et al., Tetrahedron Letters 22:1859-1862 (1981) [Exhibit 7] disclose deoxynucleoside phosphoramidites depicted in structures Ia-d and IIIa-d on page 1859 and structures Ia, II and IIIa on page 1861.

Miyoshi et al., Nucleic Acids Research 8:5491-5505 (1980) [Exhibit 8] disclose the preparation of three oligonucleotides, i.e., hexadecanucleotides in which di- and trinucleotides are used as incoming 3'-phosphodiester units. See, for example, Figure 1 on page 5493.

Gait et al., Nucleic Acids Research 8:1081-1096 (1980) [Exhibit 9] disclose the preparation of oligodeoxyribonucleotides up to 12 units long using phosphotriesters.

Duckworth et al., Nucleic Acids Research 9:1691-1706 (1981) [Exhibit 10] disclose the preparation of heptadecadeoxyribonucleotides using phosphotriesters.

Ohtsuka et al., Tetrahedron Letters 23:3081-3084 (1982) [Exhibit 11] disclose the synthesis of dodecadeoxynucleotides using phosphotriesters.

Gough et al., Tetrahedron Letters 22:4177-4180 (1981) [Exhibit 12] disclose the construction of oligodeoxyribonucleotides using phosphotriesters.

In addition to the coupling reactions disclosed in Halloran et al. cited on page 57 in the instant specification (copy attached as Exhibit 1), other procedures were known in the art prior to 1982 for coupling nucleic acid sequences to other biological polymers, including protein and polysaccharides. Among the coupling reactions known before 1982 are those listed below.

Reactions for coupling nucleic acids to other polymers (e.g., proteins, polysaccharides)

Brutlag et al., Biochemistry 8:3214-3218 (1969) [Exhibit 13] disclose cross-linking deoxyribonucleic acid to histone in nucleohistone using formaldehyde.

Manning et al., Chromosoma 53:107-117 (1975) [Exhibit 14] disclose the attachment of biotin to *Drosophila* rRNA via a cytochrome c bridge.

Politz et al., Biochemistry 20:372-378 (1981) [Exhibit 15] disclose the cross-linking of RNA to protein in *Escherichia coli* 30S ribosomal subunits using a heterobifunctional cross-linking reagent.

Cramer et al., Chemische Berichte 92:384-391 (1959) [Exhibit 16] disclose the attachment of polynucleotide sequences to polysaccharides in which 20 units of the latter was described as preferred.

ADEQUATE DESCRIPTION

10. A. It is my opinion that the above-cited portions in the specification adequately describe the presently claimed invention, including particularly those embodiments for the Sig component set forth in the claims. My opinion extends to those embodiments cited by the Examiner in the June 20, 1996 Office (page 3, first paragraph), specifically those where Sig is a moiety containing at least three carbon atoms (claims 280, 312, 340, 376, 408 and 436); Sig includes a glycosidic linkage moiety (claims 287, 319, 347, 380, 412 and 443); Sig is selected from biotin and iminobiotin (claims 288, 321, 349, 381, 413 and 449); Sig comprises ferritin (claims 289, 322, 350, 382 and 414); and Sig is selected from an antigen,

a hapten and an antibody (claims 288, 321, 349, 381, 413 and 449). It is also my opinion that the specification reasonably conveys the description that Sig may be any of the foregoing when attached to the phosphate moiety in the presently claimed nucleotides and other composition claims comprising the nucleotides because of numerous instances (nine in all!) where Sig is described as being attached to the phosphate moiety P, the sugar moiety S and/or the base moiety B. Original claim 143 is particularly significant, in my opinion, because the language specifically recites "said nucleotide having covalently attached to the P or S or B moiety a chemical moiety Sig . . ." The fact that dependent claims for the various embodiments of Sig were not included with the originally filed claims directed to the phosphate modified nucleotides (claim 141) does not in any way detract from my own conviction and opinion that the support and description for such claims would have been clearly and reasonably conveyed by reading the specification, as described in the portions cited above. It is very clear in my opinion that the specification discloses that the embodiments of Sig are to be applied - without limitation - in the disruptive and semi-disruptive positions of all three moieties recited in the independent claims, i.e., the base, sugar and phosphate moieties.

B. To elaborate further, the claimed products encompassing the various embodiments for Sig being attached to the phosphate moiety are clearly supported by the specification, particularly because all such embodiments for Sig are described as functionally equivalent for purposes of the present invention which is directed to disruptive and semi-disruptive modifications of nucleotides involving the phosphate, sugar and base moieties. The fact that one description of the phosphate-modified nucleotides is found in a paragraph that opens with "By way of summary" is of no import for at least three substantial reasons. First, as discussed above in Paragraph 9B above, there are at least nine separate instances in the specification where the attachment of Sig to any or all of the phosphate, sugar and base moieties is disclosed. The specification reasonably conveys, therefore, that the coinventors were in possession of the instantly claimed embodiments for Sig in the phosphate-modified nucleotides and compositions at the time this application was originally filed in 1982. Second, the paragraph beginning with "By way of summary" itself specifically discloses that "[t]he nucleotides are then modified in accordance with the practices of this invention by having covalently attached thereto, to the P moiety and/or the S moiety and/or the B moiety, a chemical moiety Sig." That recitation also reasonably conveys that the coinventors were in

possession of the instantly claimed subject matter at the time the application was first filed in 1982 because the specification clearly informs the reader that Sig can be attached to any of the three moieties in the nucleotide - and even to more than one moiety at the same time. Third, in the subsequent three paragraphs (page 96, last paragraph, through page 98, first paragraph) that describe embodiments for Sig, at least two instances occur where Sig is described as being attached to the phosphate, sugar or base moieties of the nucleotide:

> . . . the Sig component could comprise any chemical moiety which is attachable either directly or through a chemical linkage or linker arm to the nucleotide, such as the base B component therein, or the sugar S component therein, or the phosphoric acid P component thereof.
> [page 97, first full paragraph]

> . . . the Sig component or chemical moiety of nucleotides of this invention can be directly covalently attached to the P, S or B moieties or attached thereto via a chemical linkage or linkage arm . . .
> [page 98, first paragraph]

One could reasonably conclude from reading the three paragraphs describing the embodiments for Sig that each are applicable to not only the base moiety, but the phosphate moiety (and the sugar moiety) as well.

C. All of the foregoing reasons supports my conclusion that the specification reasonably conveys that the coinventors were in possession of the subject matter now being claimed.

D. As explained above in Paragraphs 9C and 9D, numerous reactions were known in the art for modifying the phosphate moiety of a nucleotide. It is my opinion that the specification as filed originally in 1982 reasonably conveys that the coinventors were in possession of the subject matter now being claimed and that that impression would not and does not require recitation of the litany of "phosphate-Sig" reactions indicated by the Examiner in the June 25, 1996 and June 20, 1997 Office Actions. Phosphate-Sig reactions were known in the art and an explicit description of such known reactions would not have been necessary to convey the impression that the coinventors were in possession of the subject matter now being claimed.

ENABLEMENT

11. A. It is also my opinion that the specification provides an enabling disclosure for all of the pending claims in this application. As discussed above in Paragraph 9C, Example V in the specification (page 57) provides a means for labeling the oxygen or the phosphorus of a nucleotide. As also noted above in Paragraph 9D, the chemistry and reactions for attaching substituents to the oxygen or phosphorus atoms in a nucleotidyl phosphate or phosphoric acid moiety were already known in the art at the time the initial application was filed in 1982. Although listed above after Paragraph 9D, the known chemistry and reactions are listed below for the sake of completeness.

Chemistry/Reaction	Citation	Description
oxygen	Goody et al. (1971) [Exhibit 2]	diphenyl addition to oxygen of nucleoside di- & triphosphates
oxygen	Eckstein (1975) [Exhibit 3]	methyl, aminoethyl, acetylaminoethyl & phenyl added to GDP & GTP
oxygen	Armstrong et al. (1976) [Exhibit 4]	methyl & phenyl attached to ATP & UTP analogs
phosphorus	Miller et al. (1979) [Exhibit 5]	methylene addition to prepare dideoxyribo-nucleoside methylphos-phonates
phosphorus	Miller et al. (1981) [Exhibit 6]	methylene addition to prepare dideoxyribo-nucleoside methylphos-phonates
phosphorus	Beaucage (1981) [Exhibit 7]	preparation of deoxynucleoside phosphoramidites
phosphorus	Miyoshi et al. (1980) [Exhibit 8]	oligonucleotide synthesis using phosphotriesters
phosphorus	Gait et al. (1980) [Exhibit 9]	oligodeoxynucleotide preparation using phosphotriesters

phosphorus	Duckworth et al. (1981) [Exhibit 10]	heptadecadeoxyribonucleotide preparation using phosphotriesters
phosphorus	Ohtsuka et al. (1982) [Exhibit 11]	dodecadeoxynucleotide preparation using phosphotriesters
phosphorus	Gough et al. (1981) [Exhibit 12]	oligodeoxyribonucleotide preparation using phosphotriesters
coupling	Halloran et al. (1966) [Exhibit 1]	coupling biotinylated poly-L-lysine to mono- and oligonucleotides
coupling	Brutlag et al. (1969) [Exhibit 13]	DNA to histone
coupling	Manning et al. (1975) [Exhibit 14]	biotin to rRNA using cytochrome c bridge
coupling	Politz et al. (1981) [Exhibit 15]	RNA to ribosomal protein
coupling	Cramer (1959) [Exhibit 16]	polynucleotide sequences to polysaccharides

B. It is my opinion that the subject matter now being claimed in this application, claims 278-453, could have been practiced in 1982 - with minimal experimentation and not with excessive experimentation - from a reading of the specification, particularly Example V, and further in light of the chemistry and reactions known at that time. The known chemistry and reactions are illustrated by the scientific publications cited in this Declaration above (Exhibits 1-16).

Lastly, although not an issue of enablement or adequate description, I should point out that none of the publications submitted in this Declaration (Exhibits 1-16) disclose or suggest the instantly claimed invention in which a Sig moiety is attached to the phosphate moiety of a nucleotide and which is capable of non-radioactive detection when so attached and further, is capable of incorporation into other compositions, including an oligo- or polynucleotide.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false

statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that any such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Nov. 24, 1997
Date

Dean L. Engelhardt

* * * * * * *